



Non-Invasive Evaluation of Corneal Abnormalities Using Static and Dynamic Light Scattering

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Prepared for
Photonics West
sponsored by the International Society for Optical Engineering
San Jose, California, January 19–25, 2002

National Aeronautics and
Space Administration

Glenn Research Center

Acknowledgments

NASA's Office of Physical and Biological Research and NEI/NIH-NASA Interagency Agreement 2001.
LASIK was attempted by Ronald R. Krueger, M.D. of the Cole Eye Institute, Cleveland Clinic, Cleveland, Ohio.

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ABSTRACT

A preliminary study of corneal abnormalities in intact bovine eyes is presented. Twenty-one eyes were treated with chemicals, cotton swabs, and radial and photo-refractive surgeries. Dynamic and static light scattering was performed as a function of the penetration depth into the corneal tissue. Topographical maps of corneal refractive power from untreated and treated corneas were also obtained using videokeratoscopy and results compared. The ultimate aim is to develop the technique of dynamic light scattering (DLS) for clinical applications in early evaluation of corneal complications after laser-assisted in situ keratomileusis (LASIK) surgeries and other corneal abnormalities. Keywords: LASIK, Dynamic Light Scattering, Photo Refractive Surgery, Photon Correlation Spectroscopy, Cornea

1. INTRODUCTION

Modern photo refractive surgeries, such as LASIK (Laser-assisted in situ keratomileusis), have become popular to treat corneal refractive errors. These relatively fast and apparently “routine” procedures are being performed in shopping malls across the country. Over a million procedures were estimated to have been performed in year 2001 in the United States. It has also been reported by Armour and Appleby¹ (2001) that about 5% patients suffer from complications after a LASIK procedure. The goal of refractive surgery is to sculpt the corneal surface by changing its physical shape. If successful, it will result in the elimination of overall refractive errors. Radial Keratotomy (RK), Photo refractive Keratectomy (PRK), and LASIK are three methods for performing corneal refractive surgery. RK and PRK procedures are now the least used in ophthalmic practice thus leaving LASIK as the most common procedure. PRK and LASIK methods differ in techniques but are similar in nature since they employ ablation of the cornea with laser light. In LASIK, first a mechanical cutter (Keratome) is employed to create a flap by cutting the epithelium and superficial stroma followed by the ablation of the stroma (see Figure 1). The flap is then replaced leaving epithelial layer undamaged. In ~5% post-LASIK cases patients experience a variety of effects such as haze, glare, star bursts, dry-eye syndrome, and tissue healing issues. Lipner² (2002) reports dry eye

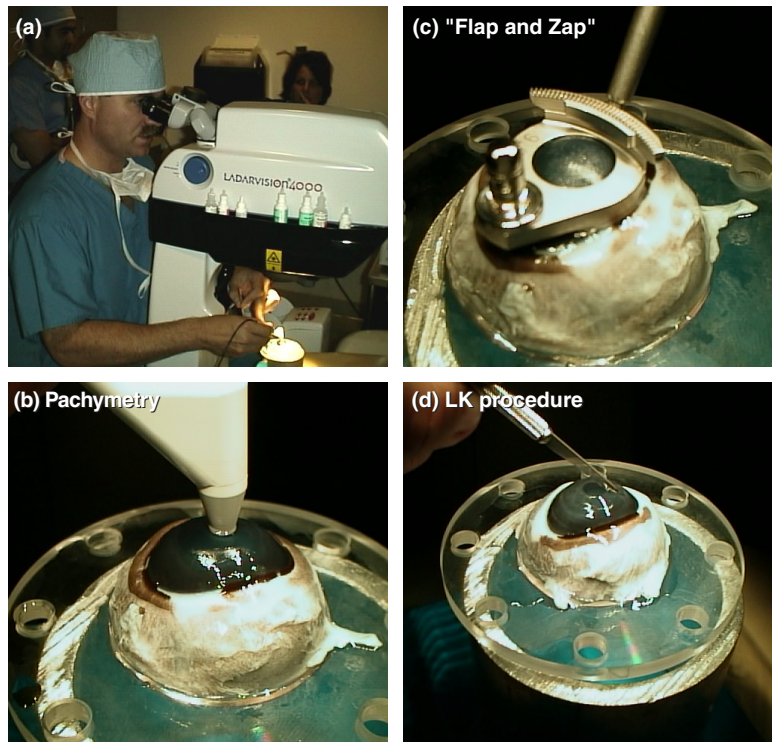


Figure 1.—In-vitro LASIK Procedure on a bovine eye. Ronald R. Krueger, M.D. of the Cole Eye Institute performing the procedure.

to be LASIK's most common complication, appearing, according to some statistics, in more than half the cases performed in the U.S. At present, no objective methods are available to evaluate quantitatively and non-invasively underlying molecular changes resulting in these corneal abnormalities after a LASIK procedure. McLeod³ (2001), in his editorial, stresses the need for new diagnostic capabilities to better evaluate current refractive surgery outcomes. Ansari and Datiles⁴ (1999), Sebag et al.⁵ (1999), Datiles et al.⁶ (2002), and Ansari et al.,⁷ (2002) have used a compact fiber optic DLS device, initially developed at NASA for fluid physics experiments in space, to detect early lens and vitreous abnormalities, e.g., cataract and diabetic vitreopathy by evaluating the molecular structures of these tissues and fluids.⁴⁻⁷ Fankhauser II and colleagues in an ARVO abstract⁸ (1997) suggested the use of DLS in detecting wound healing complications after excimer laser surgery. However, a literature search by one of us (ABL) did not reveal any published work in this area to study post-LASIK complications. The aim of this paper is to establish the experimental basis of DLS for the early and non-invasive detection of corneal abnormalities to help pave the way for upcoming clinical evaluation and monitoring of patients before and after LASIK surgeries.

1.1 Corneal Elements in a Light Scattering Experiment

The cornea is an avascular tissue. It is a typical extra-cellular matrix composed primarily of collagen. It has a refractive power of ~40 diopters (D). The entire eye has a refractive power of ~60 D. The human cornea is about 500 microns thick at the apex and about 700 microns at the periphery. It is the most sensitive structure to external insults and age-related changes⁹ (Oyster, 1999). Clearly, transparency is the most important corneal property to maintain good quality vision. Slight loss of transparency can cause problems such as haze and glare and a change in its physical shape can lead to myopic, astigmatic, and hyperopic vision. Physically, cornea can be seen as a stack of lamellar layers divided into five distinct regions. These include the tear film, epithelial cell layer, Bowman's layer, stromal layer, Descemet's membrane, and the endothelial cell layer. The tear film ~7 microns in thickness prevents the cornea from dehydration. The epithelial layer ~50 microns thick is a layer of 6-8 layers of cells consisting of 2-3 layers of superficial flat cells followed by squamous, irregular shape wing

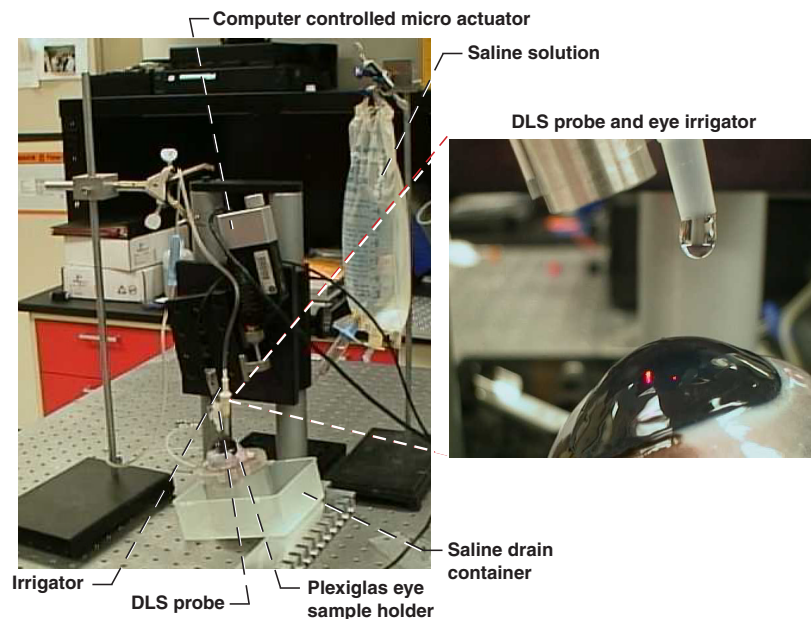


Figure 2.—Experimental setup.

(~2 layers), and cylindrical single layer basal cells. The Bowman's layer ~10 microns in thickness is like a membrane of dense irregular meshwork of interwoven collagen fibrils of type-I and type-VII without a structure (no fibroblasts). The stromal layer constitutes 90% of the thickness and volume of the cornea consisting of ~200 uniform diameter collagen fibrils with regular spacing. Fibroblasts are interposed in these collagen layers. The Descemet's membrane is a tough, glassy layer which can vary in thickness from ~5-15 microns as a function of age. The endothelium consists of a single layer of metabolically active irregular polygon shaped cells of ~20 micron diameter.

2. EXPERIMENTAL METHODS

The methods employed in this study include the techniques of dynamic light scattering (DLS) and videokeratoscopy (VK). We used a DLS fiber optic probe described elsewhere.⁴⁻⁷ The VK (Keratron corneal analyzer, model Optikon 2000, Roma, Italy) was performed to obtain a refractive power topographical map of the corneal surface. The map shows corneal regions in a 3 mm central zone in which the refractive power remains constant. The DLS experimental setup shown in Figure 2 comprises a laser, detector, the sample and probe holders, the fiber optic probe, and the data acquisition system. A semiconductor laser (Melles Griot 561MS009, ($\lambda = 666$ nm, Power = 80 microWatts), an avalanche photodiode based photon counting module (Perkin Elmer Model SPCM-AQR-14) was used as the photodetector, a DLS probe built at NASA for both static and dynamic configurations (scattering angle 163 degrees, focal length = 16 mm), and a Pentium based computer (DELL Optiplex GXi) containing a digital correlator card (Brookhaven Instrument, Model BI 9000) was used for DLS data acquisition. The DLS probe was directed into the central cornea (visual axis) with a computer-controlled micro actuator (Newport Model Motion Master 2000) to obtain anterior-posterior measurements. The time correlation functions (TCF) were collected in linear time scale with experiment duration of 10 seconds. A 1 KHZ twin-tee electronic filter was constructed to channel the correlator output to a multi-channel signal analyzer (Stanford model SR 785) to study the intensity fluctuation spectra. The bovine eyes employed in this study were obtained from an abattoir and used within 2-4 hours of sacrifice. The mild to severe abnormalities were induced by treating bovine eyes with ethanol, HCl, cotton swabs, and radial and photo-refractive surgeries. The test matrix is shown in Table 1. The tissues were kept moist by continuous drips of saline drops (1 drop every 10-15 seconds) over the cornea by using a specially designed fixture shown in Figure 2. The HCl-treated eyes involved treatment by 2-3 drops of HCl every 15 minutes for about 1 hour at an HCl concentration (w/v) of ~8%. During this time the eye was not irrigated with saline solution. Similar procedure was adopted for ethanol-treated eyes.

TABLE 1: Test Matrix (scanning steps: 10 microns/sec.)

Total Eyes	Animal	Treatment	Type	Static Scan	DLS Scan	Topographical Mapping
21	Bovine	None (control)	Excised	x	x	2,6,17,20-21
8	Guinea pig	None (control)	In-Vivo	x	x	
8	Rat	None (control)	In-Vivo	x	x	
3	Bovine	Acetone	Excised	x	x	
3	Bovine	Ethanol	Excised	x	x	x
1	Bovine	Isopropyl alcohol	Excised	x	x	
6	Bovine	Hydrochloric acid	Excised	x	x	x
2	Bovine	Cotton swab	Excised	x	x	
1	Bovine	LASIK (PRK)	Excised	x	x	x
1	Bovine	LASIK (LK)	Excised	x	x	x

2.1 Data Analysis

DLS is routinely used to measure the transport properties of suspended particles in fluids where they freely diffuse. Light passing through such a dispersion of particles fluctuates in time. The data interpretation becomes very simple since the mathematical models based on stochastic (random motion) problems in dilute dispersions of relatively small size particles (smaller than the wavelength of light) are well developed and can be used to accurately measure diffusion coefficient and particle size and size distribution^{10–11} (Chu 1973, Berne & Pecora 1976). The normalized electric field temporal autocorrelation of the fluctuation in the scattered light amplitude can be written as

$$g^1(\tau) = \exp(-\Gamma\tau) \quad (1)$$

where τ is the delay time and Γ is the decay constant due to the translational Brownian motion of the particles in the scattering volume.

$$\Gamma = D_T q^2 \quad (2)$$

where D_T is the translational diffusion coefficient, and q is the magnitude of the scattering wave vector,

$$q = (4\pi n/\lambda)\sin(\theta/2) \quad (3)$$

where n is the refractive index of the solvent, λ is the wavelength of the incident light in vacuum, and θ is the scattering angle. Using the Stokes-Einstein relation, for spherical particles, D_T can be related to the hydrodynamic radius (R) of the particle,

$$D_T = KT/6\pi\eta R \quad (4)$$

Where $K (= 1.38 \times 10^{-23} \text{ J K}^{-1})$ is the Boltzmann's constant, T is the absolute temperature of the scattering medium, and η is the solvent viscosity. The self-beating experiments reported here measure a normalized intensity-intensity temporal autocorrelation function $g^2(\tau)$ which is related to $g^1(\tau)$ via the Siegert relation,

$$g^2(\tau) = A[1 + \beta |g^1(\tau)|^2] \quad (5)$$

where $A = \langle i \rangle^2$ is the average DC photocurrent or the baseline of the autocorrelation function, and β ($0 < \beta < 1$) is an empirical experimental constant and is a measure of the spatial coherence of the scattering geometry of the collection optics which can be related to signal-to-noise (S/N).

For simple (single exponential) systems comprising monodisperse spherical particles, the correlation data can be analyzed to calculate the relaxation time ($\Gamma^{-1} = 1/D_T q^2$). This is done by fitting equation 5 with a simple linear regression of the form.

$$\ln\{[g^2(\tau)/A] - 1\} = \ln \beta - 2\Gamma(n\Delta t) \quad (6)$$

where n is the correlator channel number and Δt is the delay time per channel ($\tau = n\Delta t$). The time decay of intensity fluctuations (Γ) is extracted as slope by constructing a time correlation function (TCF) in time domain or from a spectrum analyzer in frequency domain.

Using DLS in the cornea offers opportunities and challenges. The opportunity is in its transparency since the normal cornea does not exhibit multiple scattering of light. However, at first glance, different layers and structures within the cornea seem very challenging when interpreting DLS data. Further, the corneal tissue is fairly rigid and thus it is far from the ideal case of a dispersion containing colloidal particles. We will therefore, in this first study, refrain from interpreting corneal DLS data in terms of particle size because of many unknown quantities (local viscosity, refractive index, etc.). However, it is reasonable to report the decay constant Γ (sec^{-1}) of a TCF (equations 1-6) which represents the time relaxation values of a scatterer as it executes limited Brownian excursions about fixed average positions in the corneal tissue.

3. RESULTS AND DISCUSSION

3.1 Identification of Corneal Structure

The DLS probe used in the static mode allows scanning of the eye along the optical axis to perform a reconstruction of the different layers of the corneal structure by collecting back-scattered photons. The eye was scanned from the corneal apex to the corneal endothelium in steps of 10 microns per second. This is illustrated in Figure 3 in which the data was gathered in-vivo in a two year old normal guinea pig as part of hyperbaric oxygen (HBO)-induced cataract program in collaboration with Dr. Frank Giblin of the Eye Research Institute (Oakland University) in Michigan. It typically shows anatomical features by identifying various compartments of a guinea pig eye in vivo. In a way this is equivalent to performing "histology" non-invasively. The axial length of the guinea pig's eye was found to be 10 mm. Based on the magnitude of the scattered light intensity we are able to identify peaks due to epithelium, stroma, and endothelium. Initially the intensity rises rapidly at the cornea-air interface. It decreases in the stroma and then increases in the endothelium. The local variation in intensity within the stromal structure can be attributed to scattering of light from the uniform collagen fibrils and fibroblasts interposed at equal distances.

3.2 Brownian Spectrum of Cornea

As described above, the construction of a TCF in a DLS experiment relies heavily upon the fluctuations of scattered light in time domain. We performed an experiment on a model system of polystyrene microspheres in the size range 70 nm to 1 micron

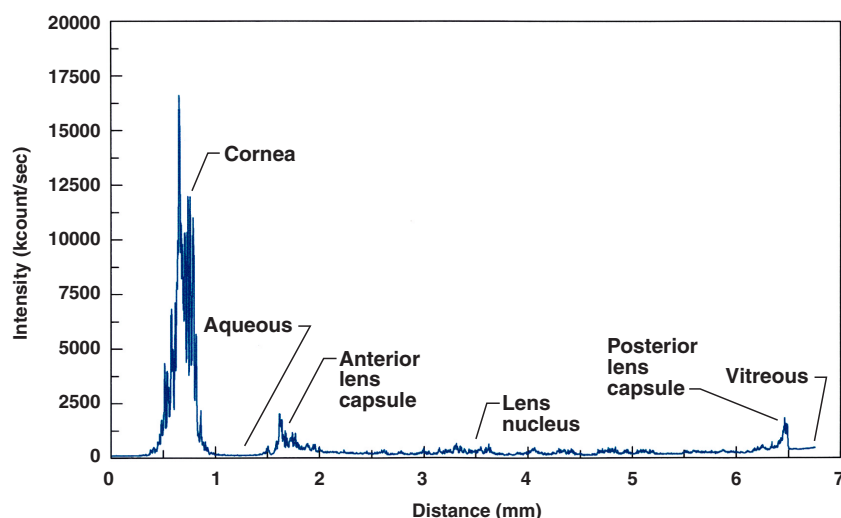


Figure 3.—In-vivo "Laser Histology": Static Light Scattering Scan showing ocular anatomical features in a guinea pig.

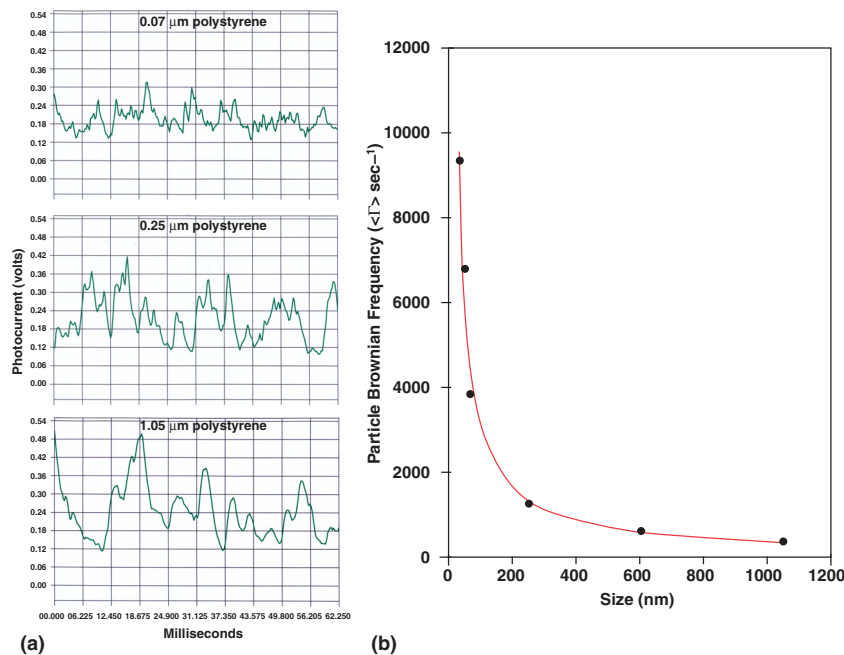


Figure 4.—Dynamic Light Scattering in model dilute polystyrene microsphere dispersions (0.006% v/v): (a) Intensity fluctuations spectrum. (b) The Brownian motion slows down as particle size increases.

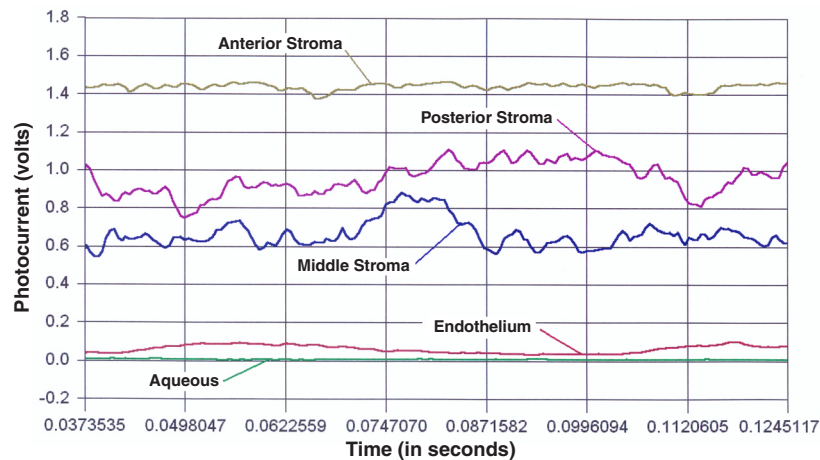


Figure 5.—Intensity Fluctuation Spectra of Bovine Cornea: Stromal layers (anterior-posterior) fluctuate more rapidly.

suspended in water and compared the time fluctuations in scattered light intensity as a function of particle size. Later, we did the same in the corneal tissue. The spectrum is illustrated in Figure 4(a) for 70 nm, 250 nm, and 1.05 μm particles. As expected, in this freely diffusing system of particles the time fluctuations gradually slows down as the particle size increases. The time decay constant (Γ) shown in Figure 4(b) obtained directly from the TCF further illustrates this point. It shows that small particles move very fast while larger particles move very slowly in a random fashion around their fixed average positions. Thus Γ becomes a powerful parameter when characterizing a system of freely diffusing and bound particles without any prior knowledge about the system (e.g., viscosity, refractive index, size, etc.). Let's compare the intensity fluctuations (Figure 5) within the layers of a normal bovine cornea. The components in the anterior stroma fluctuate slowly while in the middle and posterior sections the fluctuations are relatively faster. The endothelial cells fluctuate much slower and upon entering the

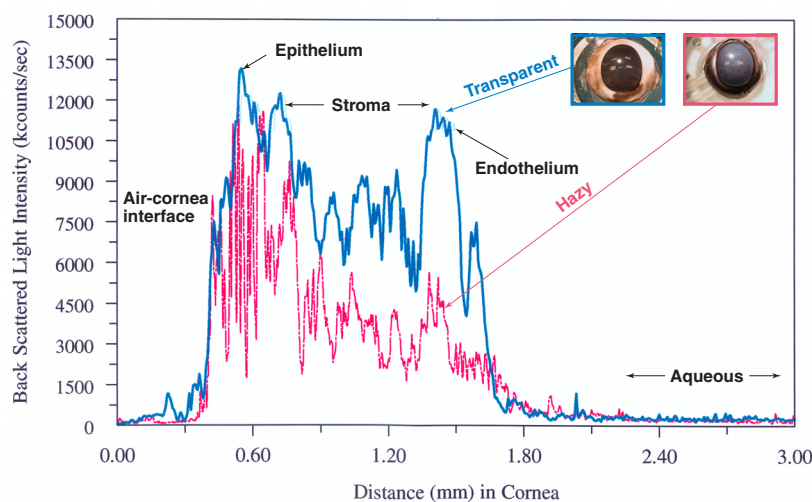


Figure 6.—Static Scans (anterior-posterior) of untreated (transparent) and ethanol-treated (hazy) bovine corneas. The markers identifying corneal layers are approximate.

aqueous the fluctuations almost disappear. The clear fluid of aqueous humor in the bovine eye hardly contains any particles or proteins of size comparable to the wavelength of the probing laser light or their concentration is extremely low to scatter light at low power levels to construct a TCF or a spectrum. The polygon shaped cells in the endothelium layer are rigidly bounded and act like a reflecting mirror so the entire structure exhibits very slow Brownian motion. The faster fluctuations in the middle and posterior stroma can be attributed to the presence of protein particles and increased amount of water. Indeed, the water content increases from anterior to posterior cornea as shown by Castoro et al.,¹² (1988) by differential scanning calorimetry. This is why the fluctuations in the anterior stroma are relatively slower. The epithelial (not shown) layer fluctuates even more slowly. In terms of post-LASIK complications, since the epithelium layer is preserved in a flap, the stromal region is of prime interest for examination with DLS.

3.3 Ethanol and Hydrochloric Acid Induced Corneal Abnormalities

Figure 6 shows a static light scattering scan of an untreated (see inset) bovine cornea. The light scattering is highest at the epithelium and gradually decreases in the stroma. First, we find the central corneal thickness to be around 0.86 mm (860 microns). The mirror-like property of the endothelium is evident with sudden rise in intensity due to polygon shaped endothelial cells. The same eye was later treated with ethanol and the results were compared. The transparent cornea slowly became hazy (see inset in Figure 6). The scan shows significant change in the corneal structure with significant reduction in the level of back-scattered photons. Another eye was scanned (not shown) after treatment with a few drops of HCl. Here we did not notice any haze but the corneal structure did change significantly as noted by reduced intensity. In Figure 7 we construct a molecular picture by plotting Γ (sec^{-1}) values obtained in a DLS measurement as a function of anterior-posterior distance. This parameter represents the frequency with which constituent molecules execute random walks around their fixed positions. In the untreated case, the lower Γ in the epithelium and Bowman's layer indicates a rigid structure of large immobile cellular particles consistent with the histology observations. The stromal data, however, is surprising. The Γ increases almost linearly indicating a less rigid structure perhaps with an increasing water content and smaller stromal corpuscles consisting of proteins in a meshwork of collagen fibrils interposed with fibroblasts. This is consistent with the discussion of section 3.2. We confirm this by presenting the TCF profiles for an untreated cornea (Figure 8) for the anterior, middle, and posterior stroma. The ethanol treated cornea shows very different results (Figures 7,9). The Γ values are lower in the anterior and middle stroma when compared with the untreated Γ values. This may be explained as follows. As ethanol evaporated the surface temperature may have dropped resulting in "freezing" or slowing down the motion of scatterers around their fixed positions. It is also possible that some ethanol may have penetrated the inner stromal layers. But we do not have any experimental evidence to support this. Similarly, the HCl-treated cornea also shows significantly different results from that of untreated cornea. The Γ values are consistently lower throughout the cornea (Figure 7). It is possible that the HCl has penetrated the

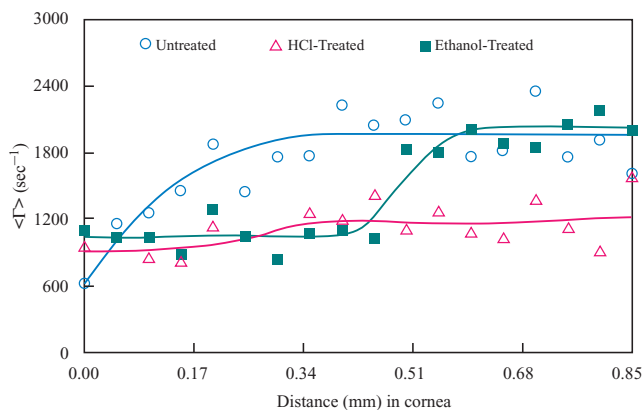


Figure 7.—Dynamic Light Scattering Measurements in untreated and HCl and ethanol-treated corneas.

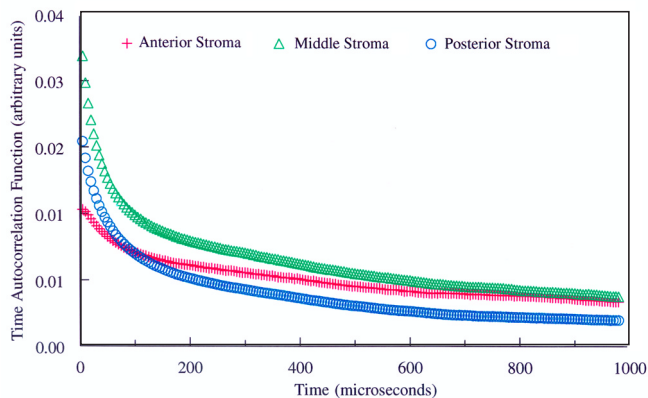


Figure 8.—TCF Profiles in the anterior, middle, and posterior corneal stroma of an untreated (normal) eye.

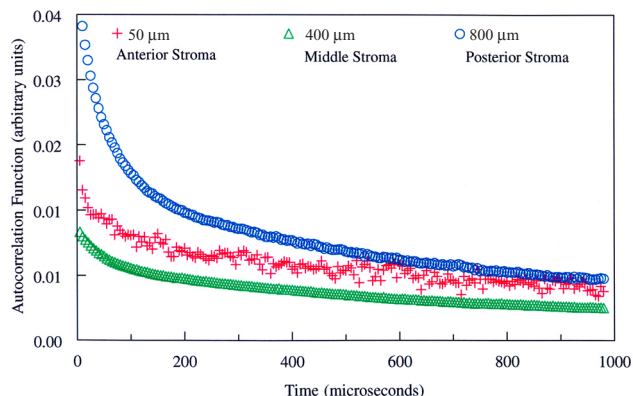


Figure 9.—TCF Profiles for ethanol-treated corneal stroma.

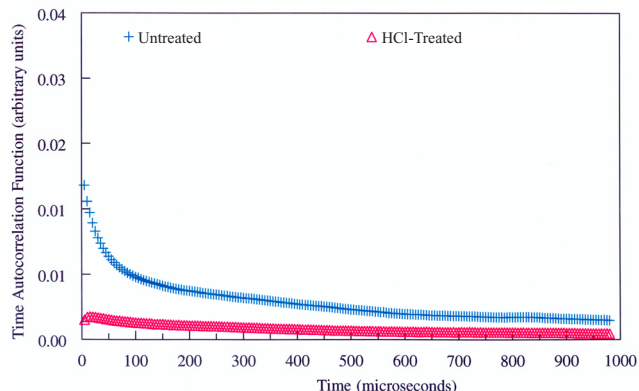


Figure 10.—TCF Profiles for the untreated and HCl-treated cornea (middle stroma).

tissue and HCl molecules have replaced the free water from the meshwork causing “freezing” of the scattering protein particles. This point is further highlighted in Figure 10 in which a TCF in the central stroma is compared for the two corneas.

3.4 Post-LASIK (PRK and LK) Cornea

Finally we present two sets of DLS data after attempting LASIK surgery on two bovine eyes in vitro. Ronald Krueger, M.D. of the Cleveland Clinic performed these procedures (see Figure 1). They are referred as eye 1 (PRK) and eye 2 (LK) and shown in Figure 11. The DLS measurements on the untreated cornea were repeated five times to get an estimate of variation (see error bars in Figure 11) in Γ values. The bovine corneas were found to be much more “rigid” than the human corneas so an attempt to make a flap using ~3 mm lamellar keratectomy was not successful. In eye 1 the epithelium was scraped and PRK (photo refractive radial keratectomy) procedure (~8 mm in size, 5.5 diopter) was performed. In eye 2 the epithelium was scraped and two lamellar keratectomies (LK) ~5 mm (no laser ablation) with a residual thickness of ~720 microns were performed. Since the corneas are reduced in size after the surgery, the DLS data for treated corneas begin at a distance of 0.2 and 0.25 mm, respectively. The two surgically treated corneas show higher Γ values compared to their untreated counterparts. The PRK and LK results differ significantly. PRK shows less increase in Γ values than LK. This is perhaps due to the loss of water during the ablation process in case of PRK. Since no laser ablation was performed in LK the Γ values remain higher. It is of interest to note that Γ values for most part revert back close to untreated values one day after the surgery. The eyes

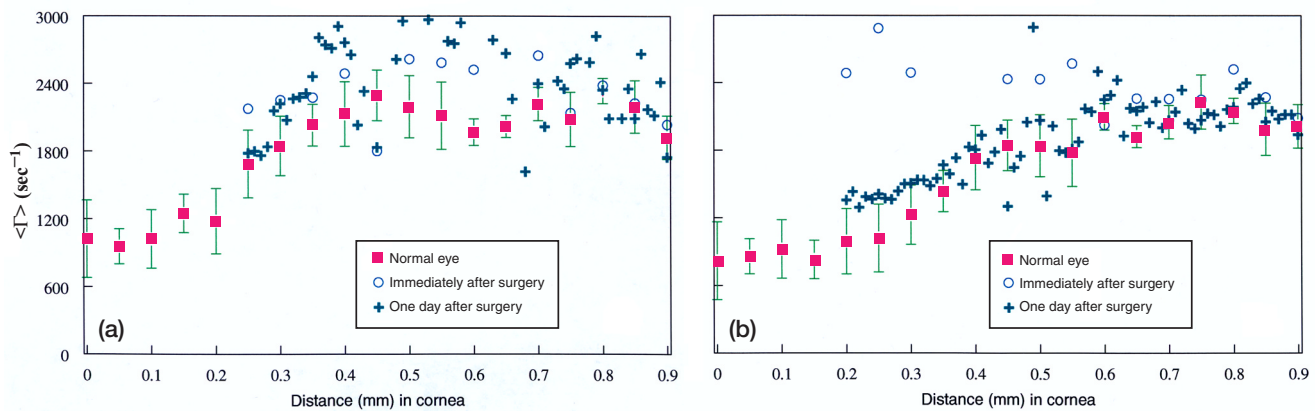


Figure 11.—Dynamic Light Scattering Measurements in untreated and treated corneas. (a) Eye 1 (PRK). (b) Eye 2 (LK).

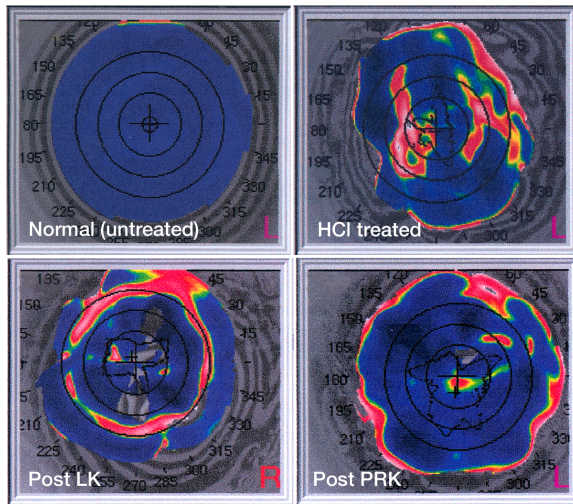


Figure 12.—Topographical Maps of untreated and treated corneas.

TABLE 2: Corneal Topographical Features

EYE	Curvature	Radius	Diopter @ Angle (3 mm zone)	Diopter change
Control (untreated)	21	16.07	25.8@ 10°	0.61
			25.2@ 100°	
HCI treated	44.35	7.61	51.48@1°	17.77
			33.7@91°	
Post-PRK surgery	72.77	4.64	42.7@62°	9.61
			33.09@152°	
Post-LK surgery	31.45	10.73	49.64@174°	22.95
			26.69@84°	

were kept in a jar of saline solution and refrigerated overnight after the surgeries. The LK procedure shows faster “healing” compared with the PRK. After corneal injury, as in surgery, the area where the epithelium has been removed is re-epithelialized by the sliding movement of the expanded remaining epithelium as well as multiplication of epithelial cells in the limbus. Two factors affecting this healing process are (1) size of the denuded area and (2) the smoothness of the denuded surface as well as presence or absence of cellular and other debris on the surface. The larger the denuded surface, the longer it takes to re-epithelialize. Since the LK surface was only 5 mm, compared to the PRK (8 mm), it healed faster. The laser-ablated (PRK) surface will still have significant debris on the surface, whereas there will be almost no debris on the lamellar keratectomized surface (LK), this difference might explain why there seems to be more healing in the LK-treated cornea. These differences can be seen by the more rapid return to normal, baseline levels of the Γ readings in the LK-treated cornea one day after the injury, compared to the PRK-treated one. However, more studies need to be performed to confirm these observations, such as using different animal models to follow the healing process using vital stains after the LK scraping of the epithelium. The treatments discussed above also changed the corneal refractive power. The results are shown in Figure 12 and Table 2. McLeod² (2001) conjectured that changes in the normal physiologic cell structure and extracellular matrix, including intracellular vacuole formation, proteoglycan content, and irregular spacing of collagen fibers, could all contribute to atypical corneal optical quality and decreased visual function. Our study clearly shows the potential of the DLS device and technique in studying these parameters safely, non-invasively, and quantitatively.

4. CONCLUSION

A preliminary study of corneal abnormalities in intact bovine eyes using the non-invasive and quantitative techniques of static and dynamic light scattering is presented. The use of DLS in the cornea, turns out to be very informative and rewarding. The experimental procedures established in this work paves the way for upcoming clinical evaluation of post-LASIK corneas at the molecular level. Exposing cornea with laser power levels of few microwatts at near infrared wavelengths in humans is very safe since the pupil dilation is not required and the focal spot rapidly defocuses past the cornea. A new instrument integrating DLS and videokeratoscopy built at NASA and already in use for cataract studies⁶ at NEI/NIH (Datiles et al., 2002) will soon be used for monitoring patients before and after LASIK surgeries.

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REPORT DOCUMENTATION PAGE			Form Approved OMB No. 0704-0188	
Public reporting burden for this collection of information is estimated to average 1 hour per response, including the time for reviewing instructions, searching existing data sources, gathering and maintaining the data needed, and completing and reviewing the collection of information. Send comments regarding this burden estimate or any other aspect of this collection of information, including suggestions for reducing this burden, to Washington Headquarters Services, Directorate for Information Operations and Reports, 1215 Jefferson Davis Highway, Suite 1204, Arlington, VA 22202-4302, and to the Office of Management and Budget, Paperwork Reduction Project (0704-0188), Washington, DC 20503.				
1. AGENCY USE ONLY (Leave blank)		2. REPORT DATE March 2002	3. REPORT TYPE AND DATES COVERED Technical Memorandum	
4. TITLE AND SUBTITLE Non-Invasive Evaluation of Corneal Abnormalities Using Static and Dynamic Light Scattering			5. FUNDING NUMBERS WU-101-51-00-00	
6. AUTHOR(S) Rafat R. Ansari, Anup K. Misra, Alfred B. Leung, James F. King, and Manuel B. Datiles III				
7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES) National Aeronautics and Space Administration John H. Glenn Research Center at Lewis Field Cleveland, Ohio 44135-3191			8. PERFORMING ORGANIZATION REPORT NUMBER E-13182	
9. SPONSORING/MONITORING AGENCY NAME(S) AND ADDRESS(ES) National Aeronautics and Space Administration Washington, DC 20546-0001			10. SPONSORING/MONITORING AGENCY REPORT NUMBER NASA TM-2002-211361	
11. SUPPLEMENTARY NOTES Prepared for Photonics West sponsored by the International Society for Optical Engineering, San Jose, California, January 19-25, 2002. Rafat R. Ansari, NASA Glenn Research Center; Anup K. Misra, Summer Intern, National Center for Microgravity Research, Cleveland, Ohio; Alfred B. Leung, Co-op Intern, Drexel University, Philadelphia, Pennsylvania; James F. King, QSS Group, Inc., Cleveland, Ohio; and Manuel B. Datiles III, National Institute of Health, Bethesda, Maryland. Responsible person, Rafat R. Ansari, organization code 6712, 216-433-5008.				
12a. DISTRIBUTION/AVAILABILITY STATEMENT Unclassified - Unlimited Subject Category: 51 Available electronically at http://gltrs.grc.nasa.gov/GLTRS This publication is available from the NASA Center for AeroSpace Information, 301-621-0390.			12b. DISTRIBUTION CODE	
13. ABSTRACT (Maximum 200 words) A preliminary study of corneal abnormalities in intact bovine eyes is presented. Twenty-one eyes were treated with chemicals, cotton swabs, and radial and photo-refractive surgeries. Dynamic and static light scattering was performed as a function of the penetration depth into the corneal tissue. Topographical maps of corneal refractive power from untreated and treated corneas were also obtained using videokeratoscopy and results compared. The ultimate aim is to develop the technique of dynamic light scattering (DLS) for clinical applications in early evaluation of corneal complications after laser-assisted in situ keratomileusis (LASIK) surgeries and other corneal abnormalities.				
14. SUBJECT TERMS LASIK; Dynamic light scattering; Photo refractive surgery; Photon correlation spectroscopy; Cornea			15. NUMBER OF PAGES 16	
			16. PRICE CODE	
17. SECURITY CLASSIFICATION OF REPORT Unclassified	18. SECURITY CLASSIFICATION OF THIS PAGE Unclassified	19. SECURITY CLASSIFICATION OF ABSTRACT Unclassified	20. LIMITATION OF ABSTRACT	